

5th International Conference on

Proteomics & Bioinformatics

September 01-03, 2015 Valencia, Spain

DNA-Affinity purification followed by mass spectrometry(AP-MS): Application to the analysis of transcriptional regulators interacting with a defined DNA sequence

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Transcription factors are targets of intense researches to better understand how cells regulate gene expression. Nonetheless, most regular assays investigating the binding of transcription factors to a DNA sequence require pre-existing knowledge about proteins supposed to bind the sequence of interest. Therefore, unbiased methods called DNA-Affinity purification followed by Mass Spectrometry (AP-MS) are being developed to identify the transcription regulators interacting with a defined DNA sequence. DNA-AP-MS has the following general scheme: Long DNA bait synthesized by PCR is immobilized on a chromatographic support and then incubated with cell nuclear extracts. The DNA-protein complexes are then eluted digested and identified by mass spectrometry. Although conceptually simple, this represents a technological challenge due to the low abundance of regulatory proteins compared to the highly abundant proteins binding to nucleic acids in a non sequence-specific manner and to the proteins adsorbed on the chromatographic support. The key parameters to increase the chances of success will be discussed such as the requirement for a specific elution step, the need for a decomplexification of the peptide sample and an adapted MS-MS protocol to focus on low abundant proteins. The crucial question of the relevant controls will also be discussed. These considerations will be illustrated through the analysis of the proteins interacting with a 226bp regulatory sequence from HIV LTR5'. Over 100 proteins were identified, among which >50% of transcription factors and co-activators/co-repressors. Some of them were functionally validated leading to a proposed model of Sin3a co-repressor complex recruitment.

Biography

Patricia Renard has completed her PhD from the University of Namur (Belgium) and Post-doctoral studies from the Pasteur Institute in Paris (France) on the activation of transcription factor NF- κ B. Beside fundamental research on cell signaling, her interest into transcription factor activation led her to develop innovative assays in this field: First an ELISA-like assay to assess the DNA-binding capacity of transcription factors and more recently, a proteomic strategy to identify transcriptional regulators after DNA-affinity purification. She is professor in the research Unit of Cell Biology of the University of Namur as well as Head of the mass spectrometry facility of this University.

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